

Animal Biosafety Level 4

These questions are based on the Animal Biosafety Level 4 section of *Biosafety in Microbiological and Biomedical Laboratories*, 3rd ed., pages 59-67.

Please circle the response that best describes the facility in which work with select agents will be carried out.

N.A. = not applicable. If you mark "N.A.", please provide a brief explanation below that item or on a separate page.

Standard Practices

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| Yes, No, N.A. | 1. | Access to the animal facility is limited or restricted at the discretion of the laboratory or animal facility director. |
| Yes, No, N.A. | 2. | Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility. |
| Yes, No, N.A. | 3. | Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in animal rooms. Persons who wear contact lenses in animal rooms should also wear goggles or a face shield. |
| Yes, No, N.A. | 4. | All procedures are carefully performed to minimize the creation of aerosols. |
| Yes, No, N.A. | 5. | Work surfaces are decontaminated after use or after any spill of viable materials. |
| Yes, No, N.A. | 6. | Doors to animal rooms open inward, are self-closing and are kept closed when experimental animals are present. |
| Yes, No, N.A. | 7. | All wastes from the animal room are appropriately decontaminated, preferably by autoclaving, before disposal. Infected animal carcasses are incinerated after being transported from the animal room in leak-proof, covered containers. |
| Yes, No, N.A. | 8. | Cages are autoclaved before bedding is removed and before they are cleaned and washed. When feasible, disposable cages that do not require cleaning are recommended; however, these cages also autoclaved before disposal. Equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility. |
| Yes, No, N.A. | 9. | An insect and rodent control program is in effect. |

Special Practices

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| Yes, No, N.A. | 1. | Only persons whose entry into the facility or individual animal room is required for program or support purposes are authorized to enter. Persons who may be at increased risk of acquiring infection or for whom infection might be unusually hazardous are not allowed in the animal facility. Persons at increased risk may include children, pregnant women, and persons who are immunodeficient or immunosuppressed. The supervisor has the final responsibility for assessing each circumstance and determining who may enter or work in the facility. Access to the facility is limited by secure, locked doors; accessibility is controlled by the animal facility supervisor, biohazards control officer, or other person responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate safeguards. Personnel comply with the instructions and all other applicable entry and exit procedures. Practical and effective protocols for emergency situations are established. |
| Yes, No, N.A. | 2. | Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially |

present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

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| Yes, No, N.A. | 3. | Baseline serum samples are collected and stored for all laboratory and other at-risk personnel. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the laboratory. The decision to establish a serologic surveillance program takes into account the availability of methods for the assessment of antibody to the agent(s) of concern. The program provides for the testing of serum samples at each collection interval and the communication of results to the participants. |
| Yes, No, N.A. | 4. | A biosafety manual is prepared or adopted. Personnel are advised of special hazards, and are required to read and to follow instructions on practices and procedures. |
| Yes, No, N.A. | 5. | When the infectious agent(s) in use in the animal room requires special entry provisions (e.g., the need for immunizations and respirators) a hazard warning sign, incorporating the universal biohazard symbol, is posted on the access door to the animal room. The hazard warning sign identifies the infectious agent(s) in use, lists the name and telephone number of the animal facility supervisor or other responsible person(s), and indicates the special requirement(s) for entering the animal room. |
| Yes, No, N.A. | 6. | Laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes. |
| Yes, No, N.A. | 7. | Hypodermic needles and syringes are used only for gavage, for parenteral injection, and aspiration of fluids from diaphragm bottles or well-restrained laboratory animals. |
| Yes, No, N.A. | a. | A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments are restricted in the laboratory for use only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware is substituted for glassware whenever possible. |
| Yes, No, N.A. | b. | Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they are carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps are placed in a hard-walled container, preferably containing a suitable disinfectant, for transport to a processing area for decontamination, preferably by autoclaving. |
| Yes, No, N.A. | c. | Syringes which re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate. |
| | d. | Broken glassware is not handled directly by hand, but is removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations. |
| Yes, No, N.A. | 8. | Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping. |
| Yes, No, N.A. | 9. | Spills and accidents which result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate, and written records are maintained. |
| Yes, No, N.A. | 10. | Personnel enter and leave the facility only through the clothing change and shower rooms. Personnel shower each time they leave the facility. Head covers are provided to personnel who do not wash their hair during the exit shower. Except in an emergency, personnel do not enter or leave the facility through the airlocks. |
| Yes, No, N.A. | 11. | Personal clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing, including undergarments, pants and shirts or jumpsuits, shoes, and gloves, are provided and used by all personnel entering the facility. When exiting, personnel remove laboratory clothing in the inner change room before entering the shower area. Soiled clothing is autoclaved before laundering. |
| Yes, No, N.A. | 12. | Supplies and materials are brought into the facility by way of a double-door autoclave, fumigation chamber, or airlock. After securing the outer doors, personnel inside the facility retrieve the materials by opening the interior door of the autoclave, fumigation chamber, or airlock. This inner door is secured after materials are |

brought into the facility. The autoclave fumigation chamber or airlock is decontaminated before the outer door is opened.

- Yes, No, N.A. 13. A system is established for the reporting of animal facility accidents and exposures, employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. An essential adjunct to such a reporting-surveillance system is the availability of a facility for the quarantine, isolation, and medical care of persons with potential or known laboratory-associated illnesses.
- Yes, No, N.A. 14. Materials (e.g., plants, animals, clothing) not related to the experiment are not permitted in the facility.

Safety Equipment (Primary Barriers)

- Yes, No, N.A. 1. Laboratory animals, infected with agents assigned to Biosafety Level 4, are housed in a Class III biological safety cabinet or in a partial containment caging system (such as open cages placed in ventilated enclosures, solid wall and bottom cages covered with filter bonnets, or other equivalent primary containment systems), in specially designed areas in which all personnel are required to wear one-piece positive pressure suits ventilated with a life support system.
- Yes, No, N.A. 2. Animal work with viral agents that require Biosafety Level 4 secondary containment, and for which highly effective vaccines are available and used, may be conducted with partial containment cages and without the one-piece positive pressure personnel suit if: the facility has been decontaminated, no concurrent experiments are being done in the facility which require Biosafety Level 4 primary and secondary containment, and all other standard and special practices are followed.

Animal Facility (Secondary Barriers)

- Yes, No, N.A. 1. The animal rooms are located in a separate building or in a clearly demarcated and isolated zone within a building. Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the facility. A double-doored autoclave, fumigation chamber, or ventilated airlock is provided for passage of materials, supplies, or equipment which are not brought into the facility through the change room.
- Yes, No, N.A. 2. Walls, floors, and ceilings of the facility are constructed to form a sealed internal shell which facilitates decontamination and is animal and insect proof. The internal surfaces of this shell are resistant to liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed.
- Yes, No, N.A. 3. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas on which dust can settle.
- Yes, No, N.A. 4. A foot, elbow, or automatically operated hand washing sink is provided in each animal room near the exit door.
- Yes, No, N.A. 5. If there is a central vacuum system, it does not serve areas outside of the facility. The vacuum system has in-line HEPA filters placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services for the facility are protected by devices that prevent backflow.
- Yes, No, N.A. 6. External animal facility doors are self-closing and selflocking.
- Yes, No, N.A. 7. Any windows must be resistant to breakage and sealed.
- Yes, No, N.A. 8. A double-doored autoclave is provided for decontaminating materials that leave the facility. The autoclave door which opens to the area external to the facility is automatically controlled so that it can only be opened after the autoclave "sterilization" cycle is completed.
- Yes, No, N.A. 9. A pass-through dunk tank, fumigation chamber, or an equivalent decontamination method is provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the facility.
- Yes, No, N.A. 10. Liquid effluents from laboratory sinks, biological safety cabinets, floor drains (if used), and autoclave chambers are decontaminated by heat treatment before being discharged to the sanitary sewer. Effluents from showers and toilets may be discharged to the sanitary sewer without treatment. The process used for

decontamination of liquid wastes must be validated physically and biologically by use of a constant recording temperature sensor in conjunction with an indicator microorganism having a defined heat susceptibility profile.

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| Yes, No, N.A. | 11. A dedicated non-recirculating ventilation system is provided. The supply and exhaust components of the system are balanced to assure directional airflow from the area of least hazard to the area(s) of greatest potential hazard. The differential pressure/directional airflow between adjacent areas is monitored and alarmed to indicate malfunction of the system. The airflow in the supply and exhaust components is monitored and the components interlocked to assure inward (or zero) airflow is maintained. |
| Yes, No, N.A. | 12. The general room exhaust air from a facility in which the work is conducted in a Class III cabinet system is treated by a passage through a HEPA filter(s) prior to discharge to the outside. The air is discharged away from occupied spaces and air intakes. The HEPA filter(s) are located as near as practicable to the source in order to minimize the length of potentially contaminated ductwork. The HEPA filter housings are designed to allow for <i>in situ</i> decontamination of the filter prior to removal, or removal of the filter in a sealed gas-tight primary container for subsequent decontamination and/or destruction by incineration. The design of the HEPA filter housing should facilitate validation of the filter installation. The use of pre-certified HEPA filters can be an advantage. The service-life of the exhaust HEPA filters can be extended through adequate filtration of the supply air. |
| Yes, No, N.A. | 13. The treated exhaust air from Class II biological safety cabinets located in a facility in which workers wear a positive pressure suit may be discharged into the animal room environment or to the outside through the facility air exhaust system. The biological safety cabinets are tested and certified at 9-month intervals. The air exhausted from Class III biological safety cabinets is passaged through two HEPA filter systems (in series) prior to discharge to the outside. If the treated exhaust is discharged to the outside through the facility exhaust system, it is connected to this system in a manner that avoids any interference with the air balance of the cabinets or the facility exhaust system. |
| Yes, No, N.A. | 14. A specially designed suit area may be provided in the facility. Personnel who enter this area wear a one-piece positive pressure suit that is ventilated by a life support system. The life support system is provided with alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker leaves the area. The exhaust air from the area in which the suit is used is filtered by two sets of HEPA filters installed in series. Duplicate filtration units and exhaust fans are provided. An automatically starting emergency power source is provided. The air pressure within the suit area is lower than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the inner shell of the suit area are sealed. A double doored autoclave is provided for decontaminating waste materials to be removed from the suit area. |